Proc. Natl. Acad. Sci. USA
Vol. 78, No. 7, pp. 4363–4367, July 1981
Cell Biology

Cytoplasmic transfer of the mitogenic response to platelet-derived growth factor

(cell fusion/cytoplasts/BALB/c 3T3 mouse cells/plasma)

J. C. SMITH AND C. D. STILES

Laboratory of Tumor Biology, Group W, Sidney Farber Cancer Institute, and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Communicated by Ruth Sager, April 6, 1981

ABSTRACT **BALB/c 3T3 mouse cells exposed briefly to platelet-derived growth factor (PDGF) become "competent" to replicate their DNA and divide. When cells are treated with PDGF and then fused to untreated cells, the resulting heterokaryons become competent to replicate their DNA. Cytoplasts derived from PDGF-treated cells are also able to transfer the growth response to untreated cells. After cytoplasmic transfer to another cell, the strength of the PDGF-induced mitogenic signal is attenuated by a factor roughly proportional to the increase in total cytoplasmic volume. When RNA synthesis is blocked during PDGF treatment, cells do not acquire the capacity to transfer the PDGF growth signal to untreated cells. By contrast, exposure to cycloheximide during PDGF treatment has no effect. These observations suggest that cytoplasmic transfer of the growth response to PDGF (competence) is mediated by a PDGF-induced stable RNA rather than by PDGF itself or a PDGF–receptor complex. The onset of DNA synthesis in PDGF–control heterokaryons occurs a minimum of 11 hr after cell fusion. Thus the substance that is transferred in these cell fusions is not directly involved in DNA synthesis; rather, it seems to trigger a sequence of events culminating in DNA synthesis.**

Platelet-derived growth factor (PDGF) is a connective tissue mitogen that has been purified to homogeneity from human platelets (1, 2). PDGF is released from platelets during clot formation; thus clotted blood serum contains PDGF. Defibrinogenated platelet-poor plasma (plasma) does not (3, 4). BALB/c 3T3 mouse cells exposed briefly to PDGF become "competent" to replicate their DNA. Competence persists for many hours after removal of PDGF from the medium; however, PDGF-treated cells make no "progress" through G_0/G_1 into S phase of growth until they are exposed to a complementary set of growth factors contained in plasma (5–7). The persistence of the PDGF effect has not been explained in molecular terms. It is possible that PDGF is ingested by receptor-mediated endocytosis (8) and remains associated with the cells; however, other growth factors such as epidermal growth factor, insulin, and the somatomedins are rapidly ingested (9–11) yet are required continually on the outside of the cell to sustain a growth response (6, 12, 13).

We have used somatic cell fusion methods to investigate the basis of the PDGF-induced competent state. Quiescent "donor" 3T3 cells are briefly exposed to PDGF. The PDGF-treated cells (or cytoplasts prepared from them) are fused to untreated "recipient" 3T3 cells. The fusion products are incubated overnight in plasma-supplemented medium (which lacks PDGF). Autoradiographic analysis of [^{3}H]thymidine uptake is used to determine whether DNA synthesis was initiated in the nucleus of the cell that was never exposed to PDGF. Colored latex beads, ingested by phagocytosis into the cell cytoplasm, are used to establish the parentage of the fusion products.

Our data suggest that PDGF stimulates the formation of a stable cytoplasmic "second signal." Formation of this second signal requires RNA synthesis. The findings are relevant to an accompanying report by Pledger *et al.* describing a set of cytoplasmic proteins induced soon after PDGF treatment; induction of these proteins requires RNA synthesis (14).

MATERIALS AND METHODS

Cell Culture. BALB/c 3T3 cells (clone A31) were maintained as described (5). All experiments were initiated on quiescent density-arrested monolayers. Fluorescein-conjugated latex beads (0.77-μm diameter) and rhodamine-conjugated latex beads (1.42-μm diameter) were from Polysciences (Warrington, PA). Density-arrested cells were incubated overnight with 1.6×10^8 fluorescein-conjugated beads per ml of growth medium or with 2.5×10^7 rhodamine-conjugated beads per ml. After overnight incubation, 90% of the cells incubated with fluorescein-conjugated beads and 78% of the cells exposed to rhodamine-conjugated beads contained 5 or more beads.

PDGF Treatment and Cell Fusion. PDGF was purified to electrophoretic homogeneity from outdated human platelets by a modification of our previous protocol (1). The pure PDGF contained 0.15 ng of protein per unit of activity (1). Because cell fusion studies consumed large quantities of PDGF, most experiments used highly purified PDGF carried through the Bio-Gel P-150 step of our protocol (1). This "Bio-Gel" PDGF was active at 10–50 ng of protein per unit. Several experiments were conducted with "crude PDGF" consisting of heat-treated platelet lysates active at 25 μg of protein per unit. The responses of 3T3 cells to crude, Bio-Gel, or pure PDGF were similar (Table 1), as has been noted previously (1, 6, 15). Platelet-poor plasma was prepared as described (5).

BALB/c 3T3 cells labeled with latex beads were exposed as indicated to PDGF or a solvent control. The two populations of cells were then harvested by trypsin digestion, resuspended in Dulbecco–Vogt modified Eagle's medium (DME medium) plus 10% plasma, and washed twice by centrifugation and resuspension. The two cell populations were mixed and plated into 60-mm culture dishes coated with collagen (TD-150, Ethicon, Somerville, MA) at 10^6 cells per dish. After 2 hr at 37°C in DME medium/10% plasma, fusion was induced with Sendai virus (Connaught Laboratories, Willowdale, ON, Canada) as described by Davidson (16). The fusion efficiency was 5–10%.

Cybrid Production. After exposure to PDGF or solvent control, cells labeled with latex beads were enucleated (17, 18) by

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: PDGF, platelet-derived growth factor; plasma, defibrinogenated platelet-poor plasma; DME medium, Dulbecco–Vogt modified Eagle's medium; DRB, 5,6-dichloro-β-D-ribofuranosylbenzimidazole.

using cytochalasin B from Aldrich. The enucleation procedure was 95% efficient and the cytoplasts retained their beads. Suspensions of intact cells labeled with latex beads were added to the cytoplast cultures at a cell-to-cytoplast ratio of 1:1 and a final density of 8×10^4 cells and cytoplasts per cm^2. After 2 hr at 37°C in DME medium/10% plasma, fusion with the cytoplasts was induced with Sendai virus (16).

Classification of Fusion Products. Cells were examined by transmitted tungsten light and reflected ultraviolet light. Binucleate cells with more than 10 beads of one color and none of the other were scored as fusion products of two cells from the same population. Binucleate cells containing more than 10 of both types of bead were scored as heterokaryons (fusion products of a PDGF and a control cell). Bead cross-contamination was measured by autoradiographic methods. The data (not shown) indicate that greater than 99% of binucleate cells with greater than 10 of both types of bead will be true heterokaryons. Criteria for identification of cybrids were established by the procedures of Jonak and Baserga (19). Cybrids were scored as mononucleate cells containing more than 10 of both types of bead. Less than 1% of such cells are nonenucleated recipients cross-contaminated with cytoplast beads. Greater than 75% of such cells have received cytoplasm from an enucleated cell.

RESULTS

Density Dependence of the Response to PDGF. PDGF controls the population density of fibroblasts in culture. At low densities, 3T3 cells can divide in medium containing little or no PDGF; at greater cell densities, high concentrations of PDGF are required for replication (20, 21). Our cell fusion studies required that PDGF-treated 3T3 cells be harvested, mixed with untreated 3T3 cells, and replated for fusion with Sendai virus. To obtain the largest difference in labeling index of cells exposed to PDGF versus untreated cells, it was necessary to determine the optimal plating density for the cell mixtures. The relationship between plating density and mitogenic response to PDGF is shown in Fig. 1. Under the standard conditions of our experiments (3- to 5-hr exposure to PDGF; subsequent incubation in DME medium/10% plasma) a plating density of 5×10^4 cells per cm^2 offers good discrimination between PDGF-treated and untreated cells. At lower cell density, the nuclear labeling index increased in untreated cells, whereas at higher cell density PDGF (at the dosages employed) induced few cells to synthesize DNA.

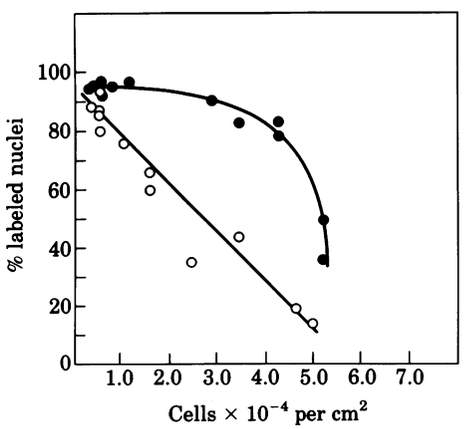

FIG. 1. DNA synthesis, after exposure to PDGF, is a function of plating density. BALB/c 3T3 cells were exposed for 4 hr to crude PDGF at 400 μg/ml (●) or to physiological saline (○). The cultures were then harvested by trypsin digestion and replated in DME medium/10% plasma containing [^{3}H]thymidine at 5 μCi/ml (1 Ci = 3.7×10^{10} becquerels). After 24 hr, cells were fixed with methanol and processed for autoradiography (5).

It is impossible always to plate out cells at exactly the same density. For this reason, there was some variation in the absolute values of the labeling indices in the cell fusion experiments below, even between replicate experiments. Therefore, for each experiment, cell counts were made from cells on the same plate. In Tables 1–5 below, valid comparisons are those made horizontally across the rows because they represent data points collected from single cultures.

PDGF-Induced Competence Is Transferred by Cell Fusion. Quiescent 3T3 cells were exposed to PDGF and then fused to untreated cells. After 24 hr incubation in DME medium/10% plasma and [^{3}H]thymidine, the cultures were fixed with methanol. Binucleate cells were classified according to their fluorescent bead content and scored for nuclear labeling by autoradiography (5). Cells were scored as labeled only if both nuclei had incorporated [^{3}H]thymidine (Fig. 2*A*). The data (Table 1) show that fusion with a PDGF-treated cell increases the probability ($P < 0.01$) that the nucleus of an untreated cell will replicate its DNA. Conversely, fusion with an untreated cell reduces the probability ($P < 0.01$) that a PDGF-treated cell will replicate its DNA; the labeling index of PDGF–control heterokaryons is consistently near the arithmetic mean of the PDGF–PDGF and control–control labeling indices. Similar results are obtained regardless of whether cells are stimulated with crude PDGF, Bio-Gel PDGF, or pure PDGF (Table 1). The absolute values of the labeling indices vary between experiments due to minor fluctuations in final cell density after fusion (see Fig. 1); however, within individual experiments, the relationship of the PDGF–PDGF, control–control, and PDGF–control labeling indices is always the same.

Cytoplasts from PDGF-Treated Cells Induce Competence. Quiescent 3T3 cells were treated with PDGF and then enucleated. The cytoplasts were fused to recipient cells that had not been treated with PDGF. After 24 hr in DME medium/10% plasma and [^{3}H]thymidine, the cultures were fixed and processed for autoradiography. Three types of mononucleate cell could be identified. These were: (*i*) untreated recipient cells that had not fused to cytoplasts, (*ii*) PDGF-treated cells that had not become enucleated (these were less than 5% of the total population), and (*iii*) cybrids (Fig. 2*B*). Labeling indices of all cell types were scored within the same culture dish. The results (Table 2) show that fusion with the cytoplast of a PDGF-treated cell increases the probability ($P < 0.01$) that an untreated cell will replicate its DNA. Control cybrids were made in which cytoplast donor cells were not exposed to PDGF. The labeling index of these control cybrids was identical to, or lower than, that of the recipient nucleated cells. Thus cybrid formation does not itself induce DNA synthesis. As in whole-cell fusion experiments (Table 1), the labeling index of the PDGF–control

Table 1. Response to PDGF is transferred by cell fusion

Exp.	% fusion products with both nuclei labeled		
	PDGF–PDGF	Control–control	PDGF–control
1	16.7	0.8	6.6
2	39.4	9.6	20.2
3	21.0	4.5	10.6

PDGF treatment was as follows: Exp. 1, crude PDGF at 700 μg/ml for 3.5 hr; Exp. 2, Bio-Gel PDGF at 8 μg/ml for 3.5 hr; Exp. 3, pure PDGF at 15 ng/ml for 4 hr. For each data point, 300 binucleate cells were scored, on the average. In all three experiments the labeling index of the control–PDGF heterokaryons is significantly greater than that of the control–control homokaryons and significantly less than that of the PDGF–PDGF homokaryons ($P < 0.01$ in all cases).

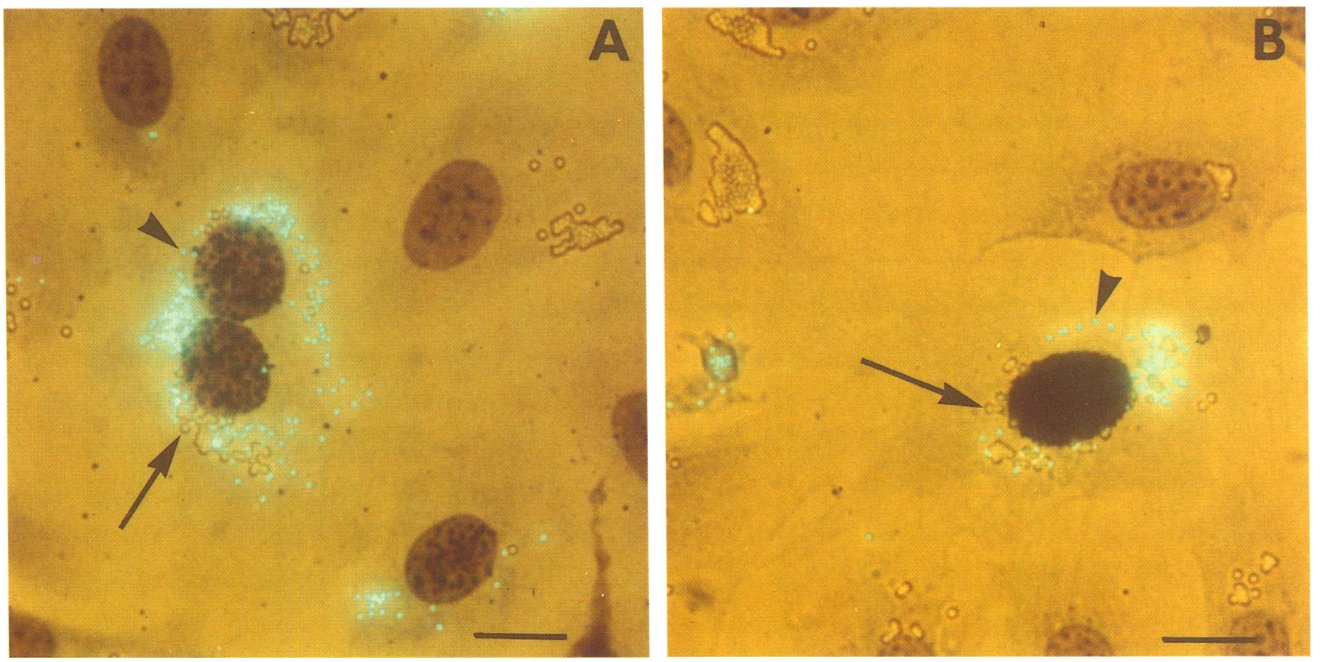

FIG. 2. Identification of heterokaryons and cybrids by fluorescent bead content. (*A*) A heterokaryon produced by fusion between a PDGF-treated cell and saline-treated control. The cytoplasm contains more than 10 fluorescein-conjugated beads (arrowhead) from the PDGF-treated cell and more than 10 rhodamine-conjugated beads (arrow) from the saline-treated cell. Both nuclei are autoradiographically labeled. (*B*) A cybrid produced by fusion of a cytoplast from a PDGF-treated cell to a control cell. The cytoplasm contains more than 10 fluorescein-conjugated beads (arrowhead) from the cytoplast and more than 10 rhodamine-conjugated beads (arrow) from the control cell. The nucleus is autoradiographically labeled. Scale bars are 20 μm.

cybrids was intermediate between the indices of PDGF-treated and untreated parents. In one experiment the labeling index of the cybrids was close to the mean of the two parents. In another it was closer to that of the untreated parent (though still significantly greater). This fluctuation probably reflects variability in the number of cytoplasts that fused to nucleated cells.

Inhibitors of RNA Synthesis Prevent Acquisition of Cytoplasmic Mitogen. Two inhibitors of RNA synthesis were tested. One inhibitor, 5,6-dichloro-β-D-ribofuranosylbenzimidazole (DRB), is reversible in action (22). The action of the other, actinomycin D, is essentially irreversible (23). In experiments with actinomycin D, cells were pretreated with the drug (2 μg/ml) for 45 min to inhibit RNA synthesis by greater than 95% (data not shown). The cells were then washed, exposed to PDGF, harvested, and fused to untreated cells. The results (Table 3) demonstrate that cells pretreated with actinomycin D do not transfer competence.

It seemed possible that the failure of actinomycin D-treated cells to transfer competence was due to internuclear exchange of actinomycin D "poisoning" the heterokaryons. To exclude this possibility, a reciprocal experiment was performed; donor cells were treated with PDGF in the absence of actinomycin D; these competent donor cells were fused to recipients that had been pretreated with actinomycin D. In these reciprocal experiments the PDGF-treated actinomycin D-treated heterokaryons had a significantly higher labeling index than the actinomycin D-treated homokaryons. Thus pretreatment of one parent cell of a heterokaryon with actinomycin D does not prevent the nucleus of the other parent from responding to PDGF.

Table 2. Response to PDGF is transferred via cytoplasts

Exp.	Pretreatment of cytoplast parent cells	% cells with labeled nuclei		
		Cytoplast parents	Recipient cells	Cybrids
1	PDGF	20.2	5.9	12.0
	Saline	2.6	3.0	3.1
2	PDGF	43.4	9.4	13.8
	Saline	10.4	12.5	8.2

On the average, 650 cells were scored for each data point. In both experiments with PDGF-treated cytoplast parents the labeling index of the cybrids is significantly greater than that of the recipient cells and less than that of the PDGF-treated cytoplast parents ($P < 0.01$). In both experiments with saline-treated cytoplast parents there are no significant differences among the labeling indices of the cybrids, the untreated recipient cells, and the cytoplast parent cells.

Table 3. Treatment with actinomycin D prevents acquisition of the PDGF response

Exp.	Actinomycin D-treated cells	% fusion products with labeled nuclei		
		PDGF–PDGF	Control–control	PDGF–control
1	PDGF	3.1	4.9	2.7
	Control	28.2	<1.5	10.4
2	PDGF	5.7	9.8	4.9
	Control	34.2	2.2	10.8

PDGF treatment was as follows: Exp. 1, crude PDGF at 700 μg/ml for 3.5 hr; Exp. 2, Bio-Gel PDGF at 50 μg/ml for 4 hr. Actinomycin D inhibits transcription by binding to DNA, preventing it from acting as a template; at high concentrations DNA replication is inhibited. In heterokaryons produced in the above experiments only the nuclei from untreated cells are generally able to synthesize DNA. Therefore, to detect transfer of competence in these experiments, binucleate cells in which only one nucleus had incorporated [^{3}H]thymidine were also scored as labeled. On the average, 200 binucleate cells were scored for each data point. In both experiments the labeling index of the control–PDGF heterokaryons is not significantly different from that of the control–control homokaryons when the PDGF-treated parent was preincubated with actinomycin D. By contrast, when the control parent was preincubated with actinomycin D, the labeling index of the control–PDGF heterokaryons is significantly greater than that of the control–control homokaryons and significantly less than that of the PDGF–PDGF homokaryons ($P < 0.01$).

Table 4. Treatment with DRB prevents acquisition of the PDGF response

Exp.	DRB-treated cells	% fusion products with both nuclei labeled		
		PDGF–PDGF	Control–control	PDGF–control
1	PDGF	17.1	15.3	16.7
	Control	22.3	2.6	9.9
2	PDGF	11.9	18.4	17.3
	Control	34.1	8.1	21.1

PDGF treatment was as follows: Exp. 1, Bio-Gel PDGF at 17 μg/ml for 3 hr; Exp. 2, Bio-Gel PDGF at 14 μg/ml for 4 hr. On the average, 225 binucleate cells were scored for each data point. In both experiments, there is no significant difference between control–control homokaryons and control–PDGF heterokaryons when the PDGF cells were treated with DRB. By contrast, in both experiments the labeling index of the control–PDGF heterokaryons is significantly greater than that of the control–control homokaryons and less than that of the PDGF–PDGF homokaryons ($P < 0.01$) when only the control parents were treated with DRB.

Similar results were obtained with DRB (15 μg/ml). This concentration of DRB inhibits total RNA synthesis by 66% in BALB/c 3T3 cells, and the effects are totally reversed within 15 min of withdrawal (data not shown). DRB preferentially inhibits synthesis of heterogeneous nuclear RNA in mammalian cells (22, 24). The data (Table 4) show that DRB at 15 μg/ml prevents 3T3 cells from responding to PDGF.

Inhibition of Protein Synthesis Does Not Prevent Acquisition of Cytoplasmic Mitogen. Quiescent 3T3 cells were preincubated with cycloheximide at 7.5 μg/ml for 15 min and then exposed simultaneously to PDGF and the same concentration of cycloheximide for 4 hr. Total protein synthesis was inhibited by 95% (data not shown). The cells were then washed and fused to recipients that had been treated neither with PDGF nor with cycloheximide. The fused cultures were processed as described for Table 1. The data indicate (Table 5) that protein synthesis is not required during exposure to PDGF for acquisition of competence.

DNA Synthesis in PDGF–Control Heterokaryons Is Initiated After an 11-hr Lag. Quiescent 3T3 cells were exposed to PDGF and fused to untreated cells. Replicate fusion cultures were fixed after intervals in DME medium/10% plasma and [^{3}H]thymidine. Autoradiographic analysis (Fig. 3) shows that PDGF–control heterokaryons entered S phase only after 11 hr. This 11-hr lag was identical to that measured for the two types of mononucleate parent cell and the two classes of binucleate homokaryons. PDGF–control heterokaryons entered S phase at a rate intermediate between the two homokaryon populations. This difference in rate of entry accounts quantitatively for the intermediate labeling indices noted previously (Tables 1 and 2).

Table 5. Treatment with cycloheximide does not prevent acquisition of the PDGF response

Exp.	% fusion products with both nuclei labeled		
	PDGF–PDGF	Control–control	PDGF–control
1	22.3	7.6	14.4
2	25.8	5.0	14.8

Cells were exposed for 4 hr to Bio-Gel PDGF at 17 μg/ml (Exp. 1) or 48 μg/ml (Exp. 2) in the presence of cycloheximide at 7.5 μg/ml. Fusion with untreated cells was then induced and the cultures were processed as described for Table 1. On the average, 330 binucleate cells were scored for each data point. In both experiments the labeling index of the control–PDGF heterokaryons is significantly greater than that of the control–control homokaryons and significantly less than that of the PDGF–PDGF homokaryons ($P < 0.01$).

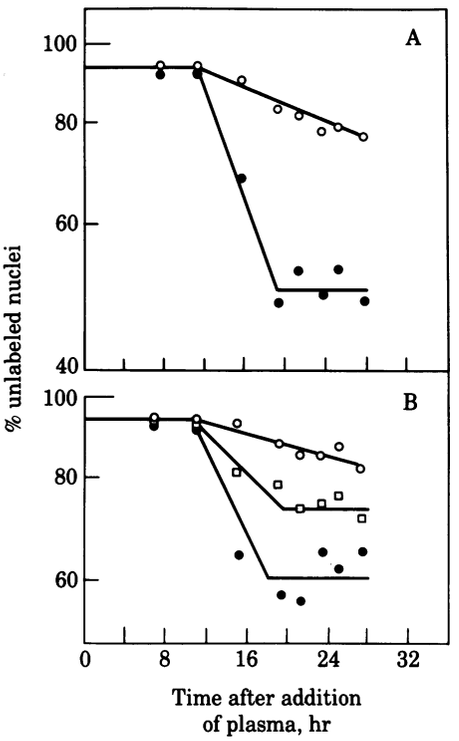

FIG. 3. Mononucleate and all classes of binucleate cells enter S phase after a lag of 11 hr. Duplicate cultures, tagged with fluorescein-conjugated or rhodamine-conjugated beads, were treated with Bio-Gel PDGF (20 μg/ml for 4½ hr) or plasma (10%). They were then washed, harvested, mixed, plated into collagen-coated culture dishes, and fused. The fused cultures were maintained in DME medium/10% plasma with [^{3}H]thymidine, fixed at the indicated intervals after plating, and processed for autoradiography. (*A*) Mononucleate cells. ●, PDGF-treated; ○, plasma-treated. (*B*) Binucleate cells. ●, PDGF–PDGF homokaryons; □, control–PDGF heterokaryons; ○, control–control homokaryons.

DISCUSSION

BALB/c 3T3 cells treated with PDGF acquire a cytoplasmic growth regulatory signal. This cytoplasmic agent initiates a mitogenic response when transferred into untreated recipient cells. When RNA synthesis is inhibited by actinomycin D or DRB during PDGF treatment, the donor cells do not acquire the cytoplasmic mitogen (Tables 3 and 4). This observation suggests that transfer of the growth response to PDGF (competence) from one cell to another does not reflect transfer of PDGF itself or a PDGF–receptor complex because RNA synthesis is not required for the initial interactions (receptor binding and receptor-mediated endocytosis) between growth factors and their target cells (25, 26). The inhibitory actions of actinomycin D and DRB do not reflect loss of a rapidly turning over PDGF receptor protein because exposure to cycloheximide during PDGF treatment has no effect on acquisition of the cytoplasmic mitogen (Table 5). Metabolic inhibitor data must always be analyzed with caution; however, a simple interpretation of the data in Tables 3 to 5 is that PDGF stimulates formation of a stable RNA. This RNA, or perhaps a translation product, initiates the mitogenic response. Our findings are relevant to a recent article by Pledger *et al.* (14); these workers have found several proteins that accumulate in the cytoplasm of BALB/c 3T3 cells soon after exposure to PDGF. When inhibitors of RNA synthesis are present during exposure to PDGF these proteins do not accumulate.

Many previous studies have shown that the cytoplasm of cells undergoing DNA synthesis contains agents that promote DNA synthesis in nonreplicating cell nuclei (27–33). Our experiments

differ from these in a fundamental way. In previous experiments employing either cell fusion techniques (27, 28, 33) or *in vitro* assays (29–32), only cells at an advanced stage of the growth cycle (late G_1 or S phase) could exert an effect on quiescent nuclei. Because two experimental variables were involved—exposure to serum growth factors and an advanced position in the cell cycle—it could not be determined whether the active agents in the cytoplasm were regulatory in nature or merely enzymes, structural components, or other substances produced during the course of cell growth.

The response to PDGF (competence) can be uncoupled from cell growth because PDGF-treated cells do not progress through the cell cycle until they are exposed to plasma growth factors (5). In our experiments, both PDGF-treated and untreated cells were quiescent and density-arrested when fusion occurred. Heterokaryons between PDGF-treated and untreated cells entered S phase after a minimum lag of 11 hr (Fig. 3); this is similar to the lag time observed when quiescent mononucleate 3T3 cells are stimulated to proliferate by the addition of fresh PDGF and plasma (Fig. 3) or whole clotted blood serum (5). Because the lag time until S phase is not shorter than usual in these fusion experiments, the agent that is transferred from the PDGF-treated cells is not involved directly in DNA synthesis: rather, it seems to trigger a sequence of events within quiescent cells that culminates in the onset of DNA synthesis. To our knowledge, such an early acting "second signal" in the mitogenic response to a growth factor has not been demonstrated previously.

This research was supported by Grants CA22427 and CA27113 from the National Cancer Institute. J.C.S. is a North Atlantic Treaty Organization Postdoctoral Fellow. C.D.S. is supported by a Faculty Research Award from the American Cancer Society.

1. Antoniades, H. N., Scher, C. D. & Stiles, C. D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1809–1813.
2. Heldin, C.-H., Westermark, B. & Wasteson, A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3722–3726.
3. Kohler, N. & Lipton, A. (1974) *Exp. Cell Res.* **87**, 297–301.
4. Ross, R., Glomset, J., Kariya, B. & Harker, L. A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1207–1210.
5. Pledger, W. J., Stiles, C. D., Antoniades, H. N. & Scher, C. D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4481–4485.
6. Stiles, C. D., Capone, G. T., Scher, C. D., Antoniades, H. N., Van Wyk, J. J. & Pledger, W. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1279–1283.
7. Stiles, C. D., Pledger, W. J., Tucker, R. W., Martin, R. G. & Scher, C. D. (1980) *J. Supramol. Struct.* **13**, 489–499.
8. Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. (1979) *Nature (London)* **279**, 679–685.
9. Carpenter, G. & Cohen, S. (1976) *J. Cell Biol.* **71**, 159–171.
10. Schlessinger, J., Schechter, Y., Willingham, M. C. & Pastan, I. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2659–2663.
11. Clemmons, D. R., Van Wyk, J. J. & Pledger, W. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6644–6648.
12. Shechter, Y., Hernaez, L. & Cuatrecasas, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5788–5791.
13. Aharanov, A., Pruss, R. M. & Herschman, H. R. (1978) *J. Biol. Chem.* **253**, 3970–3977.
14. Pledger, W. J., Hart, C. A., Locatell, K. L. & Scher, C. D. (1980) *Proc. Natl. Acad. Sci. USA* **78**, 4358–4362.
15. Tucker, R. W., Scher, C. D. & Stiles, C. D. (1979) *Cell* **18**, 1065–1072.
16. Davidson, R. (1969) *Exp. Cell Res.* **55**, 424–426.
17. Veomett, G., Shay, J., Hough, P. V. C. & Prescott, D. M. (1976) *Methods Cell Biol.* **13**, 1–5.
18. Howell, A. N. & Sager, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2358–2362.
19. Jonak, G. J. & Baserga, R. (1979) *Cell* **18**, 117–123.
20. Scher, C. D., Pledger, W. J., Martin, P., Antoniades, H. N. & Stiles, C. D. (1978) *J. Cell. Physiol.* **97**, 371–380.
21. Vogel, A., Ross, R. & Raines, E. (1980) *J. Cell Biol.* **85**, 377–385.
22. Tamm, I., Hand, R. & Caliguiri, L. A. (1976) *J. Cell Biol.* **69**, 229–240.
23. Perry, R. P. & Kelley, D. E. (1968) *J. Cell. Physiol.* **72**, 235–246.
24. Sehgal, P. B., Darnell, J. E., Jr. & Tamm, I. (1976) *Cell* **9**, 473–480.
25. Thomopoulos, P., Willingham, M. C. & Pastan, I. (1978) *Exp. Cell Res.* **116**, 478–481.
26. Carpenter, G. (1979) *J. Cell. Physiol.* **99**, 101–106.
27. Rao, P. N. & Johnson, R. T. (1970) *Nature (London)* **225**, 159–164.
28. Graves, J. A. M. (1972) *Exp. Cell Res.* **72**, 393–403.
29. Benblow, R. M. & Ford, C. C. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2437–2441.
30. Jazwinski, S. M., Wang, J. L. & Edelman, G. M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2231–2235.
31. Floros, J., Chang, H. & Baserga, R. (1978) *Science* **201**, 651–652.
32. Das, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 112–116.
33. Mercer, W. E. & Schlegel, R. A. (1980) *Exp. Cell Res.* **128**, 431–438.